Contamination of bee-collected pollens in multiple landscapes

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abstract

Honey bees are the most important managed pollinators in commercial agriculture. Large scale mixed agricultural landscapes in the mid-south are vital to maintaining commercial honey bee operations. Severe declines in bee populations have generated concerns about the role of seed-treated crops and honey bee health issues. To investigate the role of pollen in seed-treated crops as a component of bee diet, honey bee colonies were established in both agricultural and urban areas. The agricultural site was surrounded by a predominate mixture of soybeans, corn, cotton, grain sorghum and fallow fields within the foraging range of the bee hives. Urban hives were located within a large urban community garden, containing diverse fruit, vegetable and ornamental plants, as well as pasture, woodland, wild areas, and residential areas within foraging range of bee hives. Pollen traps were used to sample pollen loads directly from foraging bees, approximately every two weeks for an entire honey production season (March-September). Pollen collection began before crops were planted, and continued until crops had ceased blooming. Pollen samples were identified taxonomically to determine the proportion of the bees' pollen diet that was derived from agricultural crops for each sample. Pollen samples were also screened for pesticide residues.

introduction

As the primary managed pollinator, honey bees have a significant role in food production. When not pollinating bee-dependent crops, honey bees often depend on forage available in agricultural areas. During periods of natural nectar dearth, commercial beekeepers rely on farmland when non-cultivated landscapes are unable to support the large numbers of bee colonies required for intensive food production. Declines in honey bee populations over the last decade has raised concerns about the health and safety of bees in agricultural landscapes. Pesticides, and neonicotinoids in particular, have received much scrutiny for their perceived role in poor bee health due to their widespread use and systemic activity (Blacquière *et al.* 2012, Cresswell *et al.* 2011, Dively *et al.* 2015, Schneider *et al.* 2012).

Bees must gather all their nutrition from the surrounding landscape. Honey contains numerous enzymes and components that regulate metabolic processes in bees (Mao *et al.* 2013), but contains little nutrition, and is more fuel for adult bee activities rather than food. Pollen contains proteins, amino acids, sterols, vitamins and other nutrients vital to honey bee health and development (Di Pasquale *et al.* 2013). Both quality and quantity of pollen diversity are important, as no single pollen contains all essential amino acids to sustain bee health (Annoscia *et al.* 2017, Wright *et al.* 2017).

Modern agricultural landscapes are often large monocultures, managed heavily with herbicides that further reduce flowering plant abundance, and other pesticides potentially affecting pollinator health. The current study examines the diversity of pollen sources available in a typical agricultural landscape in the mid-south, as well as an urban setting.

materials & methods

Two study sites were selected with established apiaries. One site ("Agri") was located in Lonoke County, Arkansas, and surrounded by conventional agricultural production typical for the area (Fig. 1a). An extensive survey of the area within a 2-mile radius of the apiary during the two previous growing seasons (Johnson *et al.*, in prep) quantified land use and cultivation around the apiary (Table 1). The second site ("Urban") was adjacent to a community garden, surrounded by open pastures, woodland, and residential areas (Fig 1b). The community garden contained approximately 300 individual plots, managed independently without restrictions on what can be planted or how weeds and pests may be controlled. Some gardeners employed organic management, while others used a variety of available consumer pesticides. This site was considered representative of the environment to which area urban honey bees are routinely exposed.

Pollen traps (Brushy Mountain Bee Supply #509) were installed on three honey bee hives at each site to mechanically remove and collect pollen from bees entering the hive. Pollen was sampled for 48 hours, every two weeks, March-August 2016. This included the period before, during, and after planting treated seeds, and throughout the major bloom periods of crops. Pollen removed from each trap was immediately stored at -12 C (10° F) to preserve pesticide residues and to kill pests (*Aethina tumida* and their eggs are commonly found in area pollen traps).

All pollen from each site was pooled for each collection date and thoroughly mixed. Each sample was sorted by color to estimate plant diversity. Sorted subsamples were weighed to determine their proportion of the total bi-weekly sample. Subsamples were also examined microscopically to identify the pollen sources as crop plants (corn, cotton, soybean, grain sorghum) or non-crop (wildflower/weed) sources. Pollen identification was performed at Deschambault Animal Science Research Center, Quebec, CA. Another pooled subsample of pollen was screened for pesticide residues by the USDA-ARS National Science Laboratory, Gastonia, NC.

results

Bee colonies at the Agri site collected pollen from a total of 56 distinct plant sources during the season, while bees at the Urban site collected pollen from only 50 distinct plant sources. The proportion of each sample from crop sources is reported in Fig. 2 for the Agri site. For most collection dates, the majority of bee-collected pollen came from non-crop sources, except for period of seasonal dearth in late summer. Urban hives were not in proximity to commodity crops, however some corn was grown in community garden plots, and 2.9% of pollen sampled on June 22 was identified as corn. Results of pesticide analysis for both sites is reported in Tables 2 and 3.

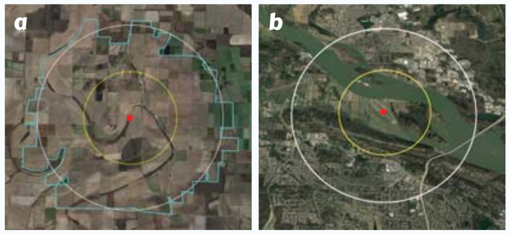

Fig 1. Landscapes of apiary sites.

Aerial maps show land use surrounding study sites, **(a)** Agri site and **(b)** Urban site. Red stars indicate bee hive locations. Yellow circles indicate a 1-mile radius from hives; white circles indicate a 2-mile radius. Blue outline on (a) indicates area surveyed for land use (see Table 1).

Table 1. Land use around agri site.

Mean acreage per crop in area surveyed during 2014-2015 growing season. Non-crop land included uncultivated fields, woodlands and natural wetlands.

crop	acres	% acreage
soybean	6,387	57.1
rice	1,099	10.0
corn	927	8.4
cotton	380	3.4
green beans	153	1.5
grain sorghum	92	2.0
total crop area	9,038	82.2
non-crop area	2,074	17.8
total acreage surveyed	11,112	100

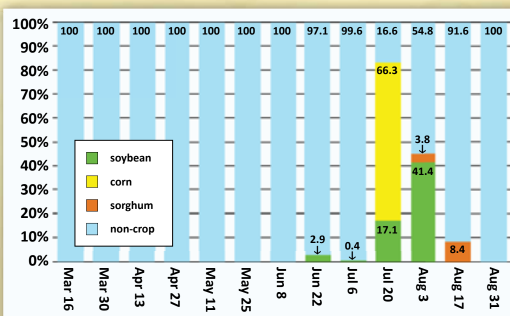

Fig 2. Sources of pollen by date.

The percentage of pollen from each crop source, collected by bees at agricultural site, is presented as a proportion of total sample collected. No pollen from cotton or rice was detected on any date. Non-crop pollen included all area wildflowers, weeds, and ornamentals. This data suggests that bees collect a majority of seasonal pollen from non-crop sources found on a small proportion of landscape surrounding agricultural area, and rely on crop plants only during seasonal nectar dearth of wild food sources.

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further information

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	compound detected	LOD	Mar 16	Mar 30	Apr 13	Apr 27	May 11	May 25	Jun 8	Jun 22	Jul 6	Jul 20	Aug 3	Aug 17	Aug 31
fungicides	azoxystrobin	2								trace		11	17		
	difenoconazole	10										10	trace		
	trifloxystrobin	10										11			
	carbendazim	5		trace	trace		trace	trace	trace			trace			7
herbicides	atrazine	4	16	16	1100	400		130	15	26	11	22	21	1100	8
	fluometuron	40					trace	trace							
	metolachlor	5			1710	238	31	56	43	41					13
	metribuzin	5			192	25	trace								
	propachlor	25											428		
	propanil	5				26	74	74	300	17		7		17	
	propazine	10				19									
insecticides	chlorantraniliprole	15									trace	36	19	30	
	cyhalothrin (total)	5										11	trace	10	
	fenpyroximate	4			trace										
	imidacloprid	1		trace											
	thiamethoxam	10			trace										

Table 2. Summary of pesticide detections from agri apiary site.

All levels reported as ppb. LOD = Level of Detection. "Trace" indicates that the compound was present, but in a concentration too low to quantify.

	compound detected	LOD	Mar 16	Mar 30	Apr 13	Apr 27	May 11	May 25	Jun 8	Jun 22	Jul 6	Jul 20	Aug 3	Aug 17	Aug 31
fungicides	azoxystrobin	2								trace					
	pyraclostrobin	15	trace												
	carbendazim	5		22	trace	trace	trace	trace							
herbi-	atrazine	4	4	5	trace	6			trace					4	
	propanil	5							11						
insecti-	carbaryl	2				3					3				
	difocol	1									71	58			
	imidacloprid	1									trace	trace			

Table 3. Summary of pesticide detections from urban apiary site.

All levels reported as ppb. LOD = Level of Detection. "Trace" indicates that the compound was present, but in a concentration too low to quantify.

conclusions

Large monoculturals are often considered poor forage for bees, and conventional pest management practices are often blamed for declines in pollinator health. This study suggests that, despite the majority of the landscape managed for a small number of crops, and extensive herbicide applications, honey bees were able to find ample resources around the study site. Liolios *et al.* (2016) concluded that bees' preference for pollen sources was not correlated to nutrient content, but was associated with its relative abundance in the landscape. Other research has suggested that bees can detect nutritional deficiencies in pollens, and actively adjust foraging to correct for deficiencies with complementary nutrients including proteins, amino acids and fatty acids (Cook *et al.* 2003, Hendriksma & Shafir 2016).

If bees simply gathered pollen in proportion to available plants, we would expect nearly half of all pollen collected during soybean bloom (mid-June through mid-August) to be from soybeans. By contrast, our data indicate that the bees in this agricultural system utilize a broad range of plant taxa from a small proportion of the land within foraging range. The brief period when bees collected a high percentage of pollen from crops coincided with a seasonal nectar dearth in Arkansas, due to lack of rain in July and August. During this period, irrigated agricultural land likely hosts the most abundant food supply for pollinators, while wild vegetation is limited.

Pesticide screening of samples provided a chronological record of potential chemical contamination of pollinator diet. Herbicides were detected throughout the season at the Agri site, reflecting their extensive use. Their detection suggests that bees consistently visited many wild plants (weeds) farmers were attempting to control, or that herbicides were drifting onto surrounding non-target vegetation, or both.

Fungicides were detected at Agri site only when bees were collecting substantial corn and soybean pollens. Levels detected here were generally low, and have low toxicity to bees, but can synergize with other compounds, particularly beekeeper-applied miticides (Johnson *et al.* 2013), and can negatively impact immune function and digestion in honey bees (Derandi-Hoffman *et al.* 2015). Fewer fungicides were detected at Urban site, although not completely absent. Herbicides were detected there at low levels, likely related to consumer products for weed control in residential lawns. Insecticide detection was also low in Urban samples.

Few insecticides were detected in samples from the Agri site. Of these, chlorantraniliprole is relatively non-toxic to honey bees, while cyhalothrins can be highly toxic (Krupke *et al.* 2014), but levels of both were very low.

Virtually all crop seeds planted in this area were treated with neonicotinoid insecticides prior to planting. However, in contrast to other reports (Krupke *et al.* 2012, Pohorecka *et al.* 2012), no quantifiable levels of these compounds were detected in pollen samples during planting periods, and no detections were made when bees were actively collecting a large proportion of crop pollen. This is consistent with a previous investigation in the same location (Stewart et al. 2014).

Overall, despite the risks that some agricultural chemicals may pose to pollinator health (Mullin et al. 2010), bee colonies in our study sites appear able to find ample nutrition in their foraging habitat, despite the highly disturbed and managed landscapes surrounding both sites. Furthermore, levels of pesticides detected in our pollen samples were not acutely toxic to bees. Further investigation is necessary to determine if any of the compounds detected pose sub-lethal risks to bees.